REMARKS

Claims 40-63 are currently pending. Claims 46, 48 and 59 are amended. Claims 62 and 63 are added. Support of these amendments can be found in the specification, for example, at page 3, lines 10-13; page 4, lines 3-4; Examples 1 and 3. None of the amendments constitute new matter.

Claims 47 and 59 are objected to for being redundant.

Claims 40-61 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Surcel *et al.* (*Immunology*, 1994, 81:171-176, "Surcel"), in view of Sørensen *et al.* (*Infection and Immunity*, 1995, 63(5):17170-1717, "Sørensen"), and Hagiwara *et al.* (*AIDS Research and Human Retroviruses*, January 20, 1996, 12(2):127-133, "Hagiwara").

I. Claim Objections

Claims 47 and 59 are objected to for being redundant. In response, claim 59 is amended to obviate this objection. In view of the amendment, Applicants respectfully request withdrawal of all claim objections.

II. Claims 40-63 Are Not Obvious Over Surcel, in view of Sørensen and Hagiwara

Claims 40-61 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Surcel, in view of Sørensen, and Hagiwara. Independent claims 40, 51 and 55 are directed to methods of diagnosis or monitoring of infection with an intracellular pathogen in an individual wherein peptide-specific or antigen-specific effector T cells are enumerated, comprising providing fresh T cells, which have not been cultured *in vitro*, in contact with a surface carrying an immobilized antibody to IFN-γ.

The Examiner contends that "one of ordinary skill in the art would have been motivated to reduce inconsistent results by using fresh T cells, rather than the cells used by Surcel that were cultured *in vitro* prior to the ELISPOT assay" because Hagiwara teaches "how the type and amount of cytokine produced *in vitro* can be altered by the culture conditions employed and that inconsistent results from such studies are not unexpected." The Examiner further contends that "[o]ne would have had a reasonable expectation of success that the use of fresh T-cells in Surcel's method would have worked because Hagiwara's method uses fresh T-cells in an ELISPOT assay." This argument relies on two erroneous suppositions: first, that the prior art results are inconsistent with respect to IFN-γ release results (the inconsistency relates instead to measuring Th1 and Th2 ratios); and second, that a person of ordinary skill in the art would have a reason to conduct IFN-γ release assays with fresh T cells in order to reduce, rather than exacerbate, the alleged "inconsistencies."

The Examiner has failed to establish a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, (1) "there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;" (2) "there must be a reasonable expectation of success;" and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations." MPEP § 2143.03.

The combined references fail to disclose or even suggest the following claim limitations:

- Presenting a T cell-activating peptide derived from an intracellular pathogen to effector T cells in a T cell-containing sample which has not been pre-cultured *in vitro*;

- Using IFN-γ release in response to stimulation with antigen derived from an intracellular pathogen as measured by ELISPOT to diagnose or monitor infection by the intracellular pathogen.

Furthermore, even if the combined references somehow taught or suggested either of these limitations, which they do not, one of ordinary skill in the art would not have combined these references with a reasonable expectation of success of achieving the instant invention.

For the reasons set forth in more detail below, the Examiner has failed to establish a *prima facie* case of obviousness.

A. The Combination Hagiwara/Surcel/Sorensen Fails to Teach or Suggest the Claimed Invention

The thrust of the subject invention is to establish a simple and reliable test for detecting the presence/absence of an intracellular pathogen in a host. This, we believe, represented an important technical problem to be solved. The state-of-the-art in 1996 (e.g. Ahmed and Gray (*Science*, 1996, 272:54-60; attached as Ex. A) put forward the idea that effector T cells are maintained only in the presence of foreign antigen; whereas memory T cells are maintained even when foreign antigen was lost from the host. The inventors for the first time appreciated that such cells might act as a "dynamic" marker for the presence/absence of an intracellular pathogen in a human host, and that detection (above background) of pathogen-specific effector T cells exclusively was an achievable objective. The various practical steps which needed to be assessed/examined in order to successfully achieve this unique goal have already been presented, and are set out again (see below).

A concept which was seminal to arrival at the invention was the realization that pathogen-specific effector T cells could be detected (above background) in an ELISPOT assay at an incubation time that is far too short for interference by memory cell progeny, *e.g.*, as little as 6 hours.

It is important to re-emphasise here that the subject invention requires the integration and interplay of seven concepts (see the Diagram summarizing these Concepts, and their interrelationship, attached as Annex I):

Concept 1 – The presence of antigen is essential to maintain population of circulating activated effector T cells *in vivo*.

Concept 2 – Effector T cells respond to antigen *in vitro* without need to proliferate or differentiate.

Concept 3 – Memory T cells only respond in vitro following proliferation (generally after 24 hours of *in vitro* stimulation with antigen).

Concept 4 – In view of Concepts 1-3 above, selective detection of pathogen-specific effector T cells could provide a dynamic surrogate marker for the presence of, or recent infection by, pathogen in a host.

Concept 5 – ELISPOT is a sensitive method, and able to enumerate responsive peptide-specific T cells *in vitro*.

Concept 6 – ELISPOT is also sufficiently sensitive to detect effector T cells above background levels before any interference from memory progeny cells occurs at later incubation times.

Concept 7 – In view of anticipated short *in vitro* incubation times, fresh cells should be introduced directly into ELISPOT wells.

Hagiwara and Sorensen teach none of the key concepts outlined above and diagrammed in Annex I. Arguably, Surcel teaches Concept 5. Therefore, Applicants submit that, as of 1996, one of ordinary skill in the art would have needed to have been in possession of at least six scientific ideas, in addition to Surcel's teaching (Concept 5), in order to gain the insight of Applicants' invention, and hence acquire the motivation to combine the above references. As a starting point, a key concept that the effector T cell could potentially provide a dynamic marker for intracellular pathogens (Concept 4) was not known to be publicly available prior to Applicants' invention. This fact is not changed by attempting to combine the teachings of Hagiwara, Surcel and Sorensen.

As explained below, the skilled artisan has no particular motivation to combine Hagiwara with the other prior art references cited by the examiner. By way of contrast, Applicants carried out the practical work necessary to prove the invention (Concepts 5-7), *because* this work was preceded by their realization that effector T cells might potentially represent a dynamic marker for the presence *in vivo* of infectious agents. This initial innovation then led to a valuable application in disease monitoring, with real practical utility.

Finally, Applicants refer briefly to Concept 7: namely "the introduction of fresh cells to the ELISPOT wells." As already noted above, Applicants maintain that Hagiwara does not teach the value of fresh cells to the skilled person. Even if this was not the case, however, and the skilled person was indeed motivated to combine Hagiwara with Surcel, the claimed invention would not have been obtained. In fact, these two citations would have collectively taught only Concepts 5 and 7 from a total of seven concepts needed to arrive at the instant invention.

Applicants set out in the instant application that the improvement offered by the subject invention is more than a predictable use of prior art elements, according to their established functions. The established purposes/functions of the three cited prior art references are:

Surcel: a comparison of T cell Th1 and Th2 profiles in PBMC in tuberculosis patients and controls;

Sorensen: a disclosure of the existence of a novel immunogenic antigen, namely ESAT-6, derived from M. tuberculosis;

Hagiwara: use of ELISPOT assays to compare the phenotype and frequency of PBMC which are spontaneously-producing cytokines,[1] including IFN-γ, in order to address the hypothesis that subjects at risk of HIV disease progression have a diminished capacity to produce Type 1 versus Type 2 cytokines.

If these purposes were to be combined, they would produce an assay methodology which explores Th1/Th2 profiles in PBMC by measuring the response of T cells to ESAT-6 mycobacterial antigen following short periods of incubation. The subject invention goes significantly beyond this combined outcome, because it focuses on the characteristics of effector T cells, and places this work into a useful clinical setting through the realisation that effector T cells specific for cognate antigens derived from a given infectious agent represent a dynamic marker for the presence/absence of that agent, and that determining the frequency of such cells can be used as an endpoint for the diagnosis and/or monitoring of infectious agents in humans.

[1] Hagiwara purports to measure "spontaneous" cytokine production, as well as that resulting from mitogen stimulation of fresh PBMCs. Presumably the mitogen stimulates cytokine expression from T cells that are poised to produce cytokine, although it is difficult to draw that conclusion since mitogen exposure would activate many other cell types (*see* Romagnani *et al.* (*Immunol. Rev.*, 1994, 140:73-92; attached as Ex. B)). Hence this work says nothing about whether the secreting cells are effector cells or other T cell phenotypes, nor does it address the antigen-specificity of the responding T cells.

It is well established that a patentable invention is not proved obvious by merely demonstrating that each of its elements was, independently, known in the prior art. *KSR Int'l. Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1741 (2007). This is what the examiner seems to be alleging. In reality, there is more to the invention than the synthesis of elements in the three cited references Hagiwara/Surcel/Sorensen, and even if it could be argued (which we contend it cannot) that the skilled person could be anticipated to combine selected aspects of these references with a reasonable expectation of success, that person would still not be in possession of key immunological knowledge needed to apply the findings to diagnosis and monitoring of the presence/absence of infectious disease. Consequently, that person would have no motivation to place the proposed prior art combination into a clinical setting.

B. The References Do Not Teach Diagnosis or Monitoring of Infection With an Intracellular Pathogen.

1. Surcel Provides No Diagnosis or Monitoring of Infection

The examiner notes (on page 4, Section 6) that "Surcel discloses Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens." Applicants concur with this view. However, the examiner goes on to say (page 8, lines 16-18) that Surcel's approach "is a method of diagnosing infection or monitoring infection." Surcel discloses no such thing. Surcel does not teach a method of diagnosis or monitoring of infection in an individual by enumerating antigen-specific IFN-γ producing T cells. Surcel compares Th1/Th2 cytokine production profiles of PBMC in response to *in vitro* stimulation of T cells by mycobacterial antigens in patients with active tuberculosis and in sensitized healthy controls. Surcel summarises a part of the results of the study by stating

that "the numbers of IFN-γ-secreting cells did not differ significantly between patients and controls" (Discussion, paragraph 2); and there was "no significant difference in IFN- γ-producing cells in response to the 38,000 MW and 19,000 MW antigens" (Summary, lines 5-7).

Absent any significant difference in the number of antigen-specific IFN-γ producing cells from patients versus controls, there is no objective basis to believe that the Surcel method could be used as a method of diagnosis or monitoring of tuberculosis infection in an individual by enumerating IFN-γ producing T cells. On the contrary, Surcel's teaching that there were no substantial differences between infected and control individuals teaches away from using this assay in diagnosis or monitoring of infection with an intracellular pathogen, e.g., tuberculosis. Where a prior art reference leads to the opposite of a limitation of the claimed invention, it cannot teach the claim limitation at issue. *See W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 U.S.P.Q. 303 (Fed. Cir. 1983), *cert. denied,*, 469 U.S. 851 (1984). This precludes predictability. *See KSR*, 127 S.Ct. at 1741. It follows that it was even less predictable that a diagnostic assay could be achieved by measuring solely effector T cells (a subcategory of T cells).

Furthermore, Surcel notes that "[c]onventionally, IFN-γ is measured in 5-7 days culture supernatants, and IFN-γ levels are found to be at least marginally decreased in TB patients." Surcel, p. 175. Thus, to the extent that Surcel provides any teaching about measuring IFN-γ levels, it motivates one of ordinary skill in the art to incubate PBMC *in vitro* for at least, and more likely considerably more than, three days before enumerating IFN-γ-secreting PBMC from patients versus controls in the hope of finding a useful diagnostic endpoint.

In summary, Surcel's results would lead a skilled person away from the idea that this approach could provide, with a reasonable expectation of success, a basis for diagnosis and monitoring of infectious disease.

2. Hagiwara Provides No Diagnosis or Monitoring of Infection

The work of Hagiwara et al. analyses the effect of HIV infection on "Type 1" and "Type 2" cytokine responses of T cells, in an effort to test the hypothesis that "a diminished capacity to produce Type 1 versus Type 2 cytokines identified subjects at risk for rapid progression of HIV disease." The work, as summarized on pages 127/128, is "focused ... on cytokine-producing cells that were actively participating in ongoing immune responses." Such cells have *already* encountered a diverse array of antigens or other activators *in vivo*, and the study therefore examined the ongoing (in Hagiwara's terms, "spontaneous") production of cytokines by these cells as measured *in vitro* in HIV-infected patients. In the process of assessing Hagiwara, a skilled person would glean the following facts:

(a) It is possible to measure, either in the absence of added stimulants (or in the presence of mitogen), spontaneously-secreting (or mitogen-activated) cytokines using short incubation times *in vitro*;

(b) Prior art studies in which cytokines were measured in supernatants following long-term incubation of PBMC with antigens/mitogens yield inconsistent results in terms of Type 1/Type 2 cytokine profiles;

(c) Hagiwara's results agree broadly with most of the prior art studies cited, in that they all report decreased IFN-γ production in HIV-infected patients versus healthy controls, and little or no difference in production as between different HIV disease stages.

Since Hagiwara's assay method is measuring immune function (*i.e.*, it distinguishes cell-mediated and humoral immune responses), it is unable to differentiate between different causes of immunodeficiency. Thus, Hagiwara is not teaching a diagnosis of HIV-AIDS; that diagnosis must be made independently and the information then correlated with immune function data.

Thus, to summarise Sections 1 and 2 above, neither Surcel nor Hagiwara provides any disclosure of diagnosing or monitoring an infection with an intracellular pathogen. For this reason alone, the obviousness rejection is in error and should be withdrawn.

3. Use of ESAT-6 in Surcel does not Anticipate the Invention

The examiner states (Page 5, Section 6) that "it would have been obvious to use ESAT-6 as the activating peptide in Surcel's ELISPOT method." Whether this is the case or not, use of ESAT-6 in Surcel's method would not bring the technical outcome any closer to the subject invention than the work already disclosed by Surcel using different mycobacterial antigens (e.g., 38,000 MW antigen), because of the absence of diagnostic or monitoring value of the Surcel assay as well as the long T cell-incubation times used by Surcel. Similarly, Sorensen used long incubation times (48 hours) for incubation of spleen cells with test antigens. Sorensen merely teaches the existence, and potential value, of another immunologically-active mycobacterial protein, and neither citation addresses the issue of whether assays designed to detect effector T cells could provide a practical basis for the diagnosis/monitoring of infection.

C. The References Do Not Teach the Diagnostic Importance of Effector T Cells

The examiner states (page 6, Section 6) that "Surcel's method is intended for measuring effector T cells." This conclusion is completely unfounded. Firstly, Surcel does not

refer to the term "effector cell" or "effector T cell," and it is evident from the long incubation times selected that both effector and memory cells will be activated. The thrust of Surcel's study is to examine Th1/Th2 profiles in tuberculosis patients and controls, and in part includes a correlation of lymphocyte proliferation and cytokine production (see Figure 3 and Table 1), with "proliferation" being detected by incorporating 3H-thymidine into lymphocyte DNA. Incorporation of this isotope is an indicator of cell division, or of the triggering of cell division.

In contrast, effector T cells are activated by cognate antigens to secrete cytokines in the absence of cell division/proliferation. Consequently, there is no support (outside of improper hindsight gained from the Applicants' disclosure) that the authors "intended" to detect effector cells. This is improper speculation that cannot form a legally sufficient basis for obviousness. It is likely that Surcel measured cytokines produced by effector T cell responses (as a component of the overall T cell response) because some test samples showed cytokine production in the absence cell proliferation, but there is no support for the view that Surcel recognizes the effector T cell as being a discrete T cell subset.

Hagiwara does not employ T cell-specific antigens, and hence provides no teaching about the *in vitro* response of any T cell type to cognate antigens.

Thus, neither Surcel nor Hagiwara (nor Sorensen) provide any disclosure leading one to select effector T cells, as recited in the claimed invention. For this reason as well, the Examiner has not established *prima facie* obviousness of the invention.

D. No Expectation of Success In Combining Surcel With Hagiwara

1. Hagiwara does not Teach Divergent Results for IFN-γ Production nor with respect to ELISPOT Assays

In the first three paragraphs of the Discussion, Hagiwara reviews some of the prior studies; and in paragraphs 2 and 3, states the following:

> Identifying alterations in immune function associated with disease progression is an important goal of HIV research. Shearer and Clerici proposed that a patient's ability to mount type 1 versus type 2 cytokine responses provided such a marker of immune function. Since reagents capable of distinguishing type 1 from type 2 cytokine-secreting cells on the basis of cell surface phenotype are not available in humans, this hypothesis was tested by stimulating cells from HIV-infected patients in vitro with mitogens and antigens. Initial results showed that disease progression was associated with a decrease in IL-2 (a type 1 cytokine) and an increase in IL-4 and IL-10 (type 2 cytokine[s]) production.
>
> Subsequent studies performed in other laboratories generally failed to support this conclusion. One group reported that the frequency of clones available to produce both type 1 and type 2 cytokines was reduced in HIV-infected individuals, while another reported no change in IFN-γ or IL-10 production following mitogen stimulation. Evidence was also presented indicating that T cell clones from HIV-infected individuals overproduced type 2 cytokines but secreted normal amounts of IFN-γ. These divergent results were generally obtained by studying PBMC that had been cultured and stimulated in vitro. Since the type and amount of cytokine produced in vitro can be altered by the culture conditions employed, inconsistent results from such studies are not unexpected.

Hagiwara, pp. 130-31 (citations omitted).

These so-called "divergent results" refer to prior inconsistent results (as between laboratories) concerning the relative levels of the collective Type 1 cytokine responses (*e.g.*, IL-2 and IFN-γ production) and collective Type 2 cytokine responses (*e.g.*, IL-4 and IL-10 production) associated with disease progression in HIV-infected patients. Although Type 1 or Type 2 cytokine responses by PBMC appear to vary due to the different culture conditions employed, Hagiwara does not teach or suggest that IFN-γ production in particular (representing

only one element of the overall Type 1/Type 2 response ratio) has been influenced by the different culture conditions employed.

With reference to IFN-γ secretion, Hagiwara reports that the number of PBMC spontaneously secreting this cytokine was significantly reduced in HIV-infected patients versus controls in an ELISPOT assay employing mitogens, not antigens, and where PBMC had not been cultured *in vitro* (Hagiwara, p. 129, Figure 3). Similar results were obtained when no stimulant was employed *in vitro* (Figure 2). In the "divergent" prior art cited by Hagiwara, Maggi *et al.* (*Science*, 1994, 265:244-48; "Maggi;" attached as Ex. C), Barcellini *et al.* (*AIDS*, 1994, 8:757-62; "Barcellini;" attached as Ex. D), Clerici *et al.* (*Science*, 1993, 262:1721-24; attached as Ex. E), and Romagnani and Maggi (*Curr. Opin. Immunol.*, 1994:4:616-22; attached as Ex. F) all report decreased IFN-γ production by PBMC from HIV-positive subjects versus HIV-negative controls after PBMC were stimulated *in vitro* by a mitogen, with or without a monoclonal antibody to CD3, for 2 or 3 days.

Hagiwara also reports no change in IFN-γ production levels amongst HIV-infected patients with differing disease severity, a result which is in agreement with the work of both Maggi and Barcellini. *See* Ex. C, D.

Thus, Hagiwara's "alternative strategy" teaches or suggests that measurement of spontaneous or mitogen-stimulated IFN-γ production in PBMC using short incubation times is broadly similar to the results in the prior art. We therefore contend that the skilled person would not identify the Hagiwara prior art results (*i.e.*, the prior art cited by Hagiwara) as "inconsistent" or "divergent" with respect to the production of IFN-γ. It is clear that the work of Hagiwara does not provide any radical insight into, or a new perspective on the prior art work, either when observing the effects of IFN-γ production by PBMC in control and HIV-infected patients, or

when comparing HIV patients in different disease states. Specifically, this concurrence between Hagiwara and the prior art provides no motivation to a skilled person to apply Hagiwara's approach, including the introducing "fresh" cells into ELISPOT wells, to assays of the type employed in the cited prior art.

Moreover, a careful analysis of the methodology employed by these prior studies reveals that the ELISPOT technique is not used as the endpoint in any of the experimental work (cited reference numbers 22, 26, 27, 39 and 40 in Hagiwara). All these references use ELISA as an endpoint to measure cytokine levels in cell-free supernatants, following long-term incubation of PBMC with antigen; and in one case (ref. 39), RIA is used as the endpoint method.

The examiner has clearly misinterpreted the inferences drawn by Hagiwara et al. regarding these prior studies by concluding that "Hagiwara teaches that ELISPOT results are divergent" (Mail Date 4/23/2008; Page 5) , and that "Hagiwara is relied upon for the teaching that ELISPOT results are divergent when studying PBMCs that have been cultured and stimulated in vitro" (Page 9).

2. Hagiwara Provides No Motivation to Modify the Surcel Method

As noted above, Hagiwara does not establish inconsistencies in IFN-γ production, or anomalies in the ELISPOT assay technique, in the cited prior art. In view of this, a skilled person, in aiming to examine T cell responses to cognate antigens using ELISPOT, would be unable to identify any one factor derived from the Hagiwara teachings which, when combined with Surcel, might provide a reasonable expectation of success such that the subject invention could be achieved. This task is rendered even more onerous by the fact that the prior art cited by Hagiwara does not, as noted above, employ ELISPOT, nor does Hagiwara's work concern cognate antigens.

Yet in spite of the above, the examiner states (on page 6, lines 9-10) that "one would have been motivated to use fresh T-cells in Surcel's method in view of Hagiwara's teachings"; and (on page 6, lines 12-13) "[g]iven this teaching one of ordinary skill in the art would have been motivated to reduce inconsistent results by using fresh T-cells, rather than the cells used by Surcel...." However, neither Hagiwara nor Surcel (nor Sorensen) disclose anything about this "problem," or the alleged solution proposed by the Examiner. "There are, in general, three possible elements giving rise to a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art." *In re Rouffet*, 149 F.3d 1350, 1357, 47 U.S.P.Q.2d 1453, 1457-58 (Fed. Cir. 1998). Without any objective basis for finding this reason (or some reason) to modify the prior art, obviousness does not obtain. *See KSR*, 127 S.Ct. at 1741. The reality is that, since Hagiwara does not point to deficiencies in either the ELISPOT technique or IFN-γ production, the skilled person would have no motivation to apply any of Hagiwara's teachings to these aspects.

3. Surcel Teaches Away from Hagiwara

Surcel teaches the use of T cells that have been cultured *in vitro* for at least 3 days. Contrary to the Examiner's statement that "Surcel's method is intended for measuring effector T-cells (active tuberculosis versus sensitized healthy controls, see Surcel's abstract)," the summary[2] of Surcel does not state that intention. Instead, it states that "[p]roliferation and cytokine production profiles by blood mononuclear cells in response to *in vitro* stimulation with mycobacterial antigens were compared in patients with active tuberculosis and in sensitized healthy controls." Surcel, p. 171, Summary. Surcel makes no suggestion that measuring effector T cells is an intended purpose. As conceded by the Examiner (page 5), "[t]he incubation of T-

[2] Surcel does not have an Abstract. Applicants assume that the Examiner intends to refer to the Summary, instead of the Abstract, in Surcel.

cells with T-cell-activating peptide for 72 hours would allow memory T-cells to proliferate, thus the measurement of IFN-gamma producing T-cells would include both the memory T cells and effector T cells;" and "[t]his measurement of both memory and effector T cells is not the instantly claimed invention's method of measuring only effector T cells." In view of the *in vitro* incubation step in the Surcel method, Surcel teaches away from the use of fresh T cells. Any inference to the contrary relies, inappropriately, on hindsight gained from Applicants' disclosure. *See W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 U.S.P.Q. 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

4. Surcel Combined With Hagiwara Is Unpredictable

When considering obviousness of a combination of known elements, the operative question is "whether the improvement is more than the predictable use of prior art elements according to their established functions." *KSR*, 127 S.Ct. at 1740. Even if one of ordinary skill in the art were motivated to modify the method of Surcel by incorporating the use of fresh PBMC or T cells as disclosed in Hagiwara, it was unpredictable that the resulting "modified" Surcel method would equate with a method of diagnosis or monitoring of infection in an individual by enumerating antigen-specific IFN-γ producing effector T cells as claimed in the present application.

The Examiner contends that "[o]ne would have had a reasonable expectation of success that the use of fresh T-cells in Surcel's method would have worked because Hagiwara's method uses fresh T-cells in an ELISPOT assay." The Examiner has ignored the major difference between the Surcel method and the Hagiwara method. While the Surcel method measures antigen-specific cytokine production, the Hagiwara method measures spontaneous cytokine production in the absence of any stimulant, or alternatively, cytokine production in

response to mitogen stimulation *in vitro*. Successful quantitation of the levels of spontaneous and/or mitogen-stimulated cytokine-producing fresh T cells suggests nothing about the likelihood of successful enumeration of *antigen-specific* cytokine-producing fresh T cells. And a combination of Surcel with Hagiwara teaches even less about the successful enumeration of antigen-specific IFN-γ producing effector cells in a sample of fresh T cells in an ELISPOT assay.

In view of the unpredictability of successful enumeration of antigen-specific IFN-γ producing fresh T cells in an ELISPOT assay, one of ordinary skill in the art would not have had a reasonable expectation that incorporation of the use of fresh T cells as disclosed in Hagiwara into the Surcel method would successfully result in a method of diagnosis or monitoring of infection in an individual by enumerating antigen-specific IFN-γ producing effector T cells. Thus, the combination was unpredictable.

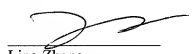
In summary, one of ordinary skill in the art would not have had any reason to modify the Surcel method by incorporating the use of fresh T cells as disclosed in Hagiwara with a reasonable expectation of success in achieving a method of diagnosis or monitoring of infection in an individual by enumerating antigen-specific IFN-γ producing effector T cells. On the contrary, each reference contains specific teachings away from such a modification. Accordingly, Applicants respectfully request withdrawal of all obviousness rejections over Surcel, in view of Sørensen and Hagiwara.

CONCLUSION

For the foregoing reasons, Applicants respectfully request that all rejections and objections be withdrawn and that all claims be allowed. Entry of the foregoing amendments and remarks into the file of the above-identified application is respectfully requested.

Applicants do not believe that any fee is due in connection with the submission of this paper. However, if any fee is due, or if any overpayment has been made, the Commissioner is authorized to charge any such fee or credit any overpayment, to our Deposit Account No. 02-4377.

Respectfully submitted,



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